b) treating said nucleic acid substrate under conditions such that said substrate forms one or more cleavage structures; and

c) reacting said endonuclease with said cleavage structures so that one or more cleavage products are produced.

54. The method of Claim 53, wherein said archael FEN-1 endonuclease comprises an *Archaeoglobus fulgidus* FEN-1 endonuclease.

55. The method of Claim 54, wherein said *Archaeoglobus fulgidus* FEN-1 endonuclease comprises SEQ ID NO:179.

56. The method of Claim 53, wherein said purified archael FEN-1 endonuclease is provided as part of a mixture, said mixture further comprising a second structure-specific nuclease, wherein said reacting said endonuclease with said cleavage structures in step c) comprises reacting said cleavage structure with said mixture.

57. The method of Claim 56, wherein said second structure-specific nuclease comprises a polymerase.

58. The method of Claim 57, wherein said polymerase comprises a thermostable polymerase.

59. The method of Claim 58, wherein said thermostable polymerase comprises a thermostable DNA polymerase altered in amino acid sequence such that it exhibits reduced DNA synthetic activity from that of a corresponding wild-type DNA polymerase but retains substantially the same 5' nuclease activity of said wild-type DNA polymerase.

60. The method of Claim 53, wherein said nucleic acid of step (a) is substantially single stranded.

61. The method of Claim 53, wherein said nucleic acid of step (a) is double stranded.

62. The method of Claim 61, wherein said treating of step (b) comprises:

i) rendering said double-stranded nucleic acid substantially single-stranded; and

ii) exposing said single-stranded nucleic acid to conditions such that said single-stranded nucleic acid has secondary structure.

A1 cont

63. The method of Claim 62, wherein said double stranded nucleic acid is rendered substantially single-stranded by the use of increased temperature.

64. The method of Claim 53, further comprising the step of detecting at least one of said one or more cleavage products.

65. A method for treating nucleic acid, comprising:

a) providing:

i) a purified FEN-1 endonuclease selected from the group consisting of *Pyrococcus woesei* FEN-1 endonuclease, *Methanococcus jannaschii* FEN-1 endonuclease, *Pyrococcus furiosus* FEN-1 endonuclease, *Methanobacterium thermoautotrophicum* FEN-1 endonuclease; and chimerical FEN-1 endonuclease; and

ii) a nucleic acid substrate;

b) treating said nucleic acid substrate under conditions such that said substrate forms one or more cleavage structures; and

c) reacting said endonuclease with said cleavage structures so that one or more cleavage products are produced.

66. The method of Claim 65, wherein said nucleic acid substrate comprises DNA.

67. The method of Claim 65, wherein said *Methanococcus jannaschii* FEN-1 endonuclease comprises SEQ ID NO:111.

68. The method of Claim 65, wherein said *Methanobacterium thermoautotrophicum* FEN-1 endonuclease comprises SEQ ID NO:183.

A1, cont

69. The method of Claim 65, wherein said *Pyrococcus furiosus* FEN-1 endonuclease comprises SEQ ID NO:115.

70. The method of Claim 65, wherein said chimerical FEN-1 endonuclease comprises at least a portion of an archael FEN-1 endonuclease.

71. The method of Claim 70, wherein said archael FEN-1 endonuclease comprises a *Archaeoglobus fulgidus* FEN-1 endonuclease.

72. The method of Claim 65, wherein said chimerical FEN-1 endonuclease comprises at least a portion of a endonuclease selected from the group consisting of *Pyrococcus woesei* FEN-1 endonuclease, *Methanococcus jannaschii* FEN-1 endonuclease, and *Methanobacterium thermoautotrophicum* FEN-1 endonuclease.

73. The method of Claim 65, wherein said purified FEN-1 endonuclease is provided as part of a mixture, said mixture further comprising a second structure-specific nuclease, wherein said reacting said endonuclease with said cleavage structures in step c) comprises reacting said cleavage structure with said mixture.

74. The method of Claim 73, wherein said second structure-specific nuclease comprises a polymerase.

75. The method of Claim 74, wherein said polymerase comprises a thermostable polymerase.

76. The method of Claim 75, wherein said thermostable polymerase comprises a thermostable DNA polymerase altered in amino acid sequence such that it exhibits reduced DNA synthetic activity from that of a corresponding wild-type DNA polymerase but retains substantially the same 5' nuclease activity of said wild-type DNA polymerase.

77. The method of Claim 65, wherein said nucleic acid of step (a) is substantially single stranded.

A1 2nd cont

78. The method of Claim 65, wherein said nucleic acid of step (a) is double stranded.

79. The method of Claim 78, wherein said treating of step (b) comprises:

i) rendering said double-stranded nucleic acid substantially single-stranded; and

ii) exposing said single-stranded nucleic acid to conditions such that said single-stranded nucleic acid has secondary structure.

80. The method of Claim 79, wherein said double stranded nucleic acid is rendered substantially single-stranded by the use of increased temperature.

81. The method of Claim 65, further comprising the step of detecting at least one of said one or more cleavage products.

82. A method for treating nucleic acid, comprising:

a) providing:

i) a first structure-specific nuclease comprising a purified FEN-1 endonuclease; and

ii) a nucleic acid substrate;

b) treating said nucleic acid substrate with increased temperature such that said substrate is substantially single-stranded;

c) reducing said temperature under conditions such that said single-stranded substrate forms one or more folded cleavage structures;

d) reacting said first nuclease with said folded cleavage structures so that one or more cleavage products are produced; and

e) detecting said one or more cleavage products.

A1 cont'd

83. The method of Claim 82, wherein said first nuclease is provided in a solution containing manganese.

84. The method of Claim 82, wherein said first nuclease is selected from the group consisting *Pyrococcus woesei* FEN-1 endonuclease, *Pyrococcus furiosus* FEN-1 endonuclease, *Methanococcus jannaschii* FEN-1 endonuclease, *Methanobacterium thermoautotrophicum* FEN-1 endonuclease, *Archaeoglobus fulgidus* FEN-1 endonuclease, and chimerical FEN-1 endonucleases.

85. The method of Claim 82, wherein said first nuclease is provided as part of a mixture, said mixture further comprising a second structure-specific nuclease, wherein said reacting said first nuclease with said folded cleavage structures in step d) comprises reacting said folded cleavage structure with said mixture.

86. The method of Claim 85, wherein said second nuclease is selected from the group consisting of the Cleavase® BN enzyme, *Thermus aquaticus* DNA polymerase, *Thermus thermophilus* DNA polymerase, *Escherichia coli* Exo III, and the *Saccharomyces cerevisiae* Rad1/Rad10 complex.

87. The method of Claim 85, wherein said second nuclease is a 5' nuclease derived from a thermostable DNA polymerase altered in amino acid sequence such that it exhibits reduced DNA synthetic activity from that of the wild-type DNA polymerase but retains substantially the same 5' nuclease activity of the wild-type DNA polymerase.

88. The method of Claim 82, wherein said nucleic acid is selected from the group consisting of RNA and DNA.

A1, cont

89. The method of Claim 82, wherein said nucleic acid of step (a) is double stranded.

90. A method for treating nucleic acid, comprising:

a) providing:

i) a purified FEN-1 endonuclease;

ii) a source of a target nucleic acid, said target nucleic acid comprising first and second portions, wherein said second portion is downstream of and contiguous to said first portion;

iii) a first oligonucleotide comprising a 5' portion complementary to said first portion of said target nucleic acid; and

iv) a second oligonucleotide comprising a 3' portion and a 5' portion, wherein said 5' portion is complementary to said second portion of said target nucleic acid;

b) generating a cleavage structure wherein at least said 5' portion of said first oligonucleotide is annealed to said first portion of said target nucleic acid and wherein at least said 5' portion of said second oligonucleotide is annealed to said second portion of said target nucleic acid; and

c) exposing said cleavage structure to said FEN-1 endonuclease to generate a cleavage product.

91. The method of Claim 90, further comprising the step of d) detecting said cleavage product.

92. The method of Claim 90, further comprising the step of generating a second cleavage structure, said second cleavage structure comprising said cleavage product hybridized to a third oligonucleotide.

93. The method of Claim 92, further comprising the step of exposing said second cleavage structure to said FEN-1 endonuclease to generate a second cleavage product.

A1 cont

94. The method of Claim 93, further comprising the step of detecting said second cleavage product.

95. The method of Claim 90, wherein said FEN-1 endonuclease comprises an archael FEN-1 endonuclease.

96. The method of Claim 95, wherein said archael FEN-1 endonuclease comprises an *Archaeoglobus fulgidus* FEN-1 endonuclease.

97. The method of Claim 96, wherein said *Archaeoglobus fulgidus* FEN-1 endonuclease comprises SEQ ID NO:179.

98. The method of Claim 90, wherein said FEN-1 endonuclease comprises *Pyrococcus woesei* FEN-1 endonuclease.

99. The method of Claim 90, wherein said FEN-1 endonuclease comprises *Methanococcus jannaschii* FEN-1 endonuclease.

100. The method of Claim 90, wherein said FEN-1 endonuclease comprises *Methanobacterium thermoautotrophicum* FEN-1 endonuclease.

101. The method of Claim 90, wherein said FEN-1 endonuclease comprises *Pyrococcus furiosus* FEN-1 endonuclease.

102. The method of Claim 90, wherein said FEN-1 endonuclease comprises a chimerical FEN-1 endonuclease.

103. The method of Claim 102, wherein said chimerical FEN-1 endonuclease comprises at least a portion of an archael FEN-1 endonuclease.

A1 concl

104. The method of Claim 103, wherein said archael FEN-1 endonuclease comprises a *Archaeoglobus fulgidus* FEN-1 endonuclease.

105. The method of Claim 102, wherein said chimerical FEN-1 endonuclease comprises at least a portion of a endonuclease selected from the group consisting of *Pyrococcus woesei* FEN-1 endonuclease, *Methanococcus jannaschii* FEN-1 endonuclease, and *Methanobacterium thermoautotrophicum* FEN-1 endonuclease.

106. The method of Claim 90, wherein said 3' portion of said second oligonucleotide comprises a 3' terminal nucleotide not complementary to said target nucleic acid.

107. The method of Claim 90, wherein said 3' portion of said second oligonucleotide consists of a single nucleotide not complementary to said target nucleic acid.

108. The method of Claim 90, wherein at least one of said first oligonucleotide and said second oligonucleotide is attached to a solid support.